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Yield components and nutritive value of *Robinia pseudoacacia* and *Albizia julibrissin* in Arkansas, USA

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Abstract Ranchers need alternative livestock feeds when herbaceous forages become limiting in summer. Our objectives were to determine: (1) leaf and stem biomass components, (2) nutritive value [in vitro dry matter digestibility (IVDMD), total nonstructural carbohydrate (TNC), N, and N digestibility] of leaves for animal browse, (3) concentration of the secondary metabolites robinin and mimosine, and (4) in vitro leaf and bark toxicity for black locust (*Robinia pseudoacacia* L.) and mimosa (*Albizia julibrissin* Durz.), respectively, pollarded at 50 cm in Arkansas, USA. Black locust exceeded mimosa for every yield component (leaf mass tree^{-1}, leaves shoot^{-1}, shoots tree^{-1}, shoot mass tree^{-1}, stem basal area, and biomass tree^{-1}) except mass leaf^{-1}. Projected yields were 1,900 and 1,600 kg leaves ha^{-1} for black locust and mimosa, respectively, assuming a population of 12,300 trees ha^{-1}. Mimosa leaves had greater IVDMD, TNC, and N digestibility than black locust. Mimosa leaves exceeded the nutritional N requirements of growing cattle (*Bos taurus* L.) and goats (*Capra hircus* L.), but protein supplementation would be needed for growing goats grazing black locust leaves. Tissue concentrations of secondary metabolites robinin and mimosine were below detectable limits in black locust and mimosa, respectively. The extract of black locust bark, but not leaves, was toxic to bioassayed African green monkey (*Cercopithecus aethiops* L.) cells. Either black locust or mimosa could provide moderate quantities of high quality, rotationally grazed forage for goats during summer months when herbaceous forage may in short supply.

Keywords Black locust · Crude protein · In vitro dry matter digestibility · Mimosa · Mimosine · Nitrogen · Pollard · Robinin

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Introduction

Black locust (subfamily *Faboideae*) and mimosa (subfamily *Mimosaceae*) are tree species in *Fabaceae*

Lindl. that could be used for livestock browse in USA and other countries. Both species are invasive in some ecosystems (Mullen et al. 2000; Rice et al. 2004), and their browse is highly preferred by goats (Luginbuhl and Mueller 1999). Goats fed black locust leaves in summer had similar live weight gain (>100 g day^{-1}) as goats fed alfalfa (*Medicago sativa* L.) pellets (Papachristou et al. 1999). Voluntary intake of alfalfa meal and black locust leaf meal did not differ for sheep (*Ovis aries* L.) in a study in which weight gain was not measured (Horton and Christensen 1981). Nutritive value analysis (Baertsche et al. 1986; Burner et al. 2005) suggests that black locust has potential as livestock feed.

The feed value of black locust leaves has been debated for some time mainly because of the presence of antiquality components. Black locust is toxic to the horse, *Equus caballus* L. (Ball et al. 1996) but not to goats (Cheeke 1992; Govt. of Canada 2006). It is not known if black locust is toxic to cattle or sheep. Data are limited or contradictory on specific leaf toxins, toxin concentrations, critical toxicity levels for ruminants, and whether cultural practice might influence toxin concentrations. Data also are limited on secondary metabolites in black locust. The nontoxic flavonoid robinin (kaempferol-3-*O*-robinoside-7-*O*-rhamnoside), a complex of isomeric forms of a triglycoside, occurs in *Robinia* spp. flowers (Maksyutina 1972; Sando 1932), but presence in leaves is not documented. Robinin has antioxidant properties that benefit human health, and could be a value-added flavonoid extracted prior to biofuel conversion (Lau et al. 2005).

Less is known about the nutritive value of mimosa than black locust. Mimosa leaves have less neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude protein (CP), and is less preferred by goats than black locust (Addlestone et al. 1999). An unidentified water insoluble, nonalkaloid constituent present in pods and seeds of at least two *Albizia* species causes cattle toxicity in southern Africa (Basson et al. 1970). Mimosine [β-N-(3-hydroxy-4-pyridone)-α-aminopropionic acid] is a toxic alkaloid present in leaves of *Leucaena* spp. (Soedarjo et al. 1994). Mimosine in *Leucaena leucocephala* decreases in concentration with leaf age, from 45 g kg^{-1} in 1-week-old to 2 g kg^{-1} in 10-week-old leaves (Tangendjaja et al. 1986). Mimosa does not appear to have been tested for mimosine despite anecdotal evidence of its presence (Williams et al. 2000; R. D. Williams, 2006, personal communication).

The objectives of this study were to determine: (1) leaf and stem biomass components, (2) nutritive value [in vitro dry matter digestibility (IVDMD), total nonstructural carbohydrate (TNC), N, and N digestibility] of leaves for animal browse, (3) quantities of the secondary metabolites robinin and mimosine, and (4) in vitro leaf and bark toxicity for black locust and mimosa trees pollarded at 50 cm in Arkansas, USA.

Materials and methods

The experiment was situated on a Linker soil (fine-loamy, siliceous, semiactive, thermic Typic Hapludult) near Booneville, Arkansas (35.11°N, 93.95°W; 152 m above sea level). Initial composition of the topsoil (15-cm depth) was pH 5.5, 15 kg P ha^{-1}, 450 kg K ha^{-1}, and 1.57 g N kg^{-1}. Fertilizer was applied to the soil surface at the rate of 55 kg P ha^{-1} and 55 kg K ha^{-1} in March 2001, and 340 kg P ha^{-1} in March 2002.

One-year-old black locust trees (Lawyer Nursery Inc., Plains, MT, USA) and 2-year-old mimosa trees (started from seed collected in Oklahoma, USA) were inoculated by dipping roots in species-specific strains of *Rhizobium* spp. (Liphatech, Milwaukee, WI, USA). Trees were transplanted in February 2002 at a spacing of 0.9 m within and between rows (12,300 trees ha^{-1}). There were four contiguous rows of each species in each plot. Trees in the center two rows were for sampling while those in the outer two rows served as buffers. Alleys were mowed periodically to control herbaceous vegetation.

Immediately after transplanting, height and basal stem diameter were measured for about 20 trees $plot^{-1}$. During establishment, dormant trees were pollarded at 50-cm height above soil surface in March 2003 and January 2004 to stimulate shoot proliferation. Dry mass of pollard clippings was measured for about 20 trees $plot^{-1}$. At this location during November through April, these species are dormant due to cold temperatures and daylength. During the study, dormant trees also were pollarded in December 2004.

A stationary weather station (Spectrum Technologies, Inc., Plainfield, IL, USA) was located 4 km west of the study site. Data including air temperature

(measured by a thermocouple at 1.5 m above soil surface), soil temperature (measured by a thermocouple placed 15 cm below soil surface under grass sod), and rainfall (measured with a tipping bucket recorder at 1.5 m above soil surface) were recorded at 0.5-h intervals in July through September 2004 and 2005. Long-term (1971–2000) air temperature and rainfall data for Booneville, AR, USA (NOAA 2002) were used for reference. Air temperature was lower during July–September 2004 than in the same months of 2005 or the long-term mean (Fig. 1a), whereas soil temperature was lower in August 2004 than in August 2005 (Fig. 1b). More rainfall was received in July 2004 and September 2005 than the long-term mean for the same months, but rainfall was similar to the long-term mean in August 2004 and 2005 (Fig. 1c).

Yield measurements

Four trees were harvested at random from each plot in July, August, and September 2004 and 2005. These months were selected because forage quality and quantity can become limiting in pastures and rangeland in the southern USA. Shoots were clipped from the main stem at 50-cm height above soil surface and counted. Basal area tree^{-1} (mm^2) was calculated as the sum of basal diameters of each clipped shoot measured 2 cm distal to the cut. Leaves (petioles and leaflets) were removed from the clipped shoots, including 5–10 cm of unlignified shoot apex. Leaves and shoots were counted and combined for the four sampled trees per plot. Leaf and shoot dry mass were measured after oven drying (60°C) for 48 and 120 h, respectively. Shoots of a particular tree were sampled only once yearly, so measurements at any given harvest date represented growth accumulated from the beginning of the growing season. Leaf and shoot mass were expressed on a dry basis (g shoot^{-1} and g tree^{-1}). Mass leaf^{-1} was calculated as the ratio of leaf dry mass to number of leaves. Harvested leaf and shoot dry mass were summed to calculate aboveground biomass tree^{-1}.

Leaf nutritive value

Leaf samples were ground to pass a 2-mm screen and stored at –20°C until analyzed. IVDMD was analyzed

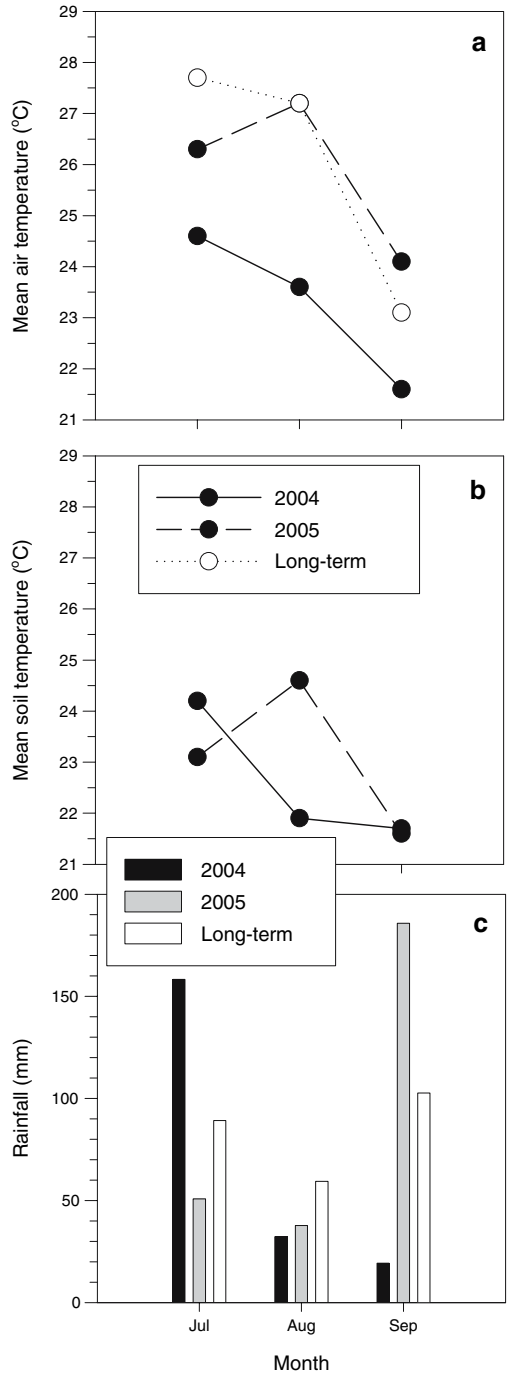

Fig. 1 **a** Mean monthly air temperature, **b** mean topsoil (15-cm depth) temperature, and **c** total rainfall for July–September in 2004 and 2005, and long-term (1971–2000) mean air temperature and total rainfall at Booneville, Arkansas (NOAA 2002)

using an ANKOM® Daisy II fiber analyzer #F200 (ANKOM Technology Corp., Fairport, NY, USA). The rumen fluid for digestibility analysis was

collected from a cannulated steer (*Bos taurus* L.) fed bermudagrass (*Cynodon dactylon* L.) hay for at least 1 week prior to collection. Leaf samples were further ground in a cyclone mill (Cyclotec 1093, Foss Tecator, Eden Prairie, MN, USA) to pass a 1-mm screen for N and TNC analyses. Nitrogen was analyzed by a total combustion method (Leco FP428, Leco Corp., St. Joseph, MO, USA). TNCs, including starch, were determined as described previously (Burner and Belesky 2004). Nitrogen digestibility was estimated for samples collected in 2004 using the equation (total N - IVDMD residual N)/total N × 100.

Robinin and mimosine analyses

A separate leaf sampling was taken at each harvest date in 2005 for measuring the concentration of robinin in black locust and mimosine in mimosa. Leaves were collected from two trees per plot, frozen on dry ice (–80°C) in the field, lyophilized at –50°C and 4 kPa for 2 days, ground to pass a 1-mm screen, and stored at –20°C until analysis. Bark (outer and inner bark) was removed from several random trees from each species in summer 2006, composited, oven dried, ground to pass a 1-mm screen, and stored at –20°C until analysis.

Leaf and bark tissues of black locust, and kudzu [*Pueraria montana* (Lour.) Merr. var. *lobata* (Willd.) Maesen & S. M. Almeida] leaves, were analyzed for robinin and compared to a purified robinin standard (Cat. No. 021032, Indofine Chemical Co., Hillsborough, NJ, USA). Kudzu leaves, provided by D. Bransby (Auburn Univ., Auburn, AL, USA), were used as a standard because of its high robinin concentration (6.5 g kg^{-1}, Lau et al. 2005). Robinin was extracted from tissues and analyzed by high-performance liquid chromatography (HPLC) as previously described (Lau et al. 2005), with 1 g tissue extracted in 30 mL methanol. Retention time was 21.4 min and robinin absorbance was quantified at 265.8 and 346.5 nm.

Leaf and bark samples of mimosa were analyzed for mimosine and compared to a purified standard (L-mimosine, Cat. No. M-003, Indofine Chemical Co.). A dilution series of L-mimosine was created in 0.4 M NaOH and 1.0 N HCl. Mimosine was extracted from tissues in 0.1 N HCl (Hegarty et al. 1964) and analyzed by HPLC. Absorbance was measured at 280 nm using a modification of the procedures by Lau et al. (2005) and Soedarjo et al. (1994). A 250 × 4.6 mm^2 Luna 5 μ C18(2) column (Phenomenex, Torrence, CA, USA) was preconditioned with 0.1% formic acid for 15 min before injecting a 10 µL sample. Mimosine absorbance was measured at 216.4 and 281.2 nm at a retention time of 3.3 min.

In vitro toxicity analysis

Bark and leaf tissues of black locust and mimosa were extracted in their respective solvents, methanol for black locust and 0.1 N HCl for mimosa. Half of the extract was evaporated to dryness under N_2 at room temperature. The dry residue was re-dissolved in 1 mL of dimethylsulfoxide (DMSO) (Mallinckrodt Baker, Phillipsburg, NJ, USA), from which 1:2, 1:4, and 1:8 (extract : distilled water) dilutions were prepared. Toxicity of the extracts was measured with the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) toxicity kit (TOX-1) from Sigma, St. Louis, MO, USA. The assay was performed as described by Mossman (1983). Vero 76 African green monkey kidney-derived cells (American Type Cell Culture, ATCC, Manassas, VA, USA) were cultured in Dulbecco's modified eagle medium (DMEM) from ATCC. The medium was modified with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA, USA) and supplemented with 1% penicillin-streptomycin-neomycin and 1% amphoterecin B (Sigma). Each microplate well (flat-bottomed, 96-well Costar, VWR Int., West Chester, PA, USA) received 1×10^5 cells in DMEM and 10 µL of the extract. The negative control was 10 µL Triton® X-100 (Calbiochem, La Jolla, CA, USA), and the positive control was 10 µL of DMSO. Plates were incubated for 18 h at 37°C in a CO_2 incubator (model 2325, VWR Int.). After incubation, MTT (prepared as specified by the manufacturer) was added to each well and incubated for an additional 2 h before adding the MTT solubilizing solution (10% Triton X-100 plus 0.1 N HCl in anhydrous isopropanol). The plates were read in a microplate reader (BioTek, Winooksi, VT, USA). The background absorbance at 690 nm was subtracted from the measured absorbance at 570 nm. Cellular viability was calculated as [(sample absorbance - positive

control)/(positive control - negative control)] × 100. This was a test of cellular toxicity, but probably a poor predictor of ruminant toxicity because of the potential detoxifying activity of certain rumen bacteria (Hammond 1995).

Statistical analysis

Mean monthly air temperature and total monthly rainfall were computed for July through September 2004 and 2005, and compared to the long-term mean (NOAA 2002). The field experiment was a randomized complete block design with three replications. Analysis of variance (ANOVA) used a mixed linear model, Proc Mixed (Littell et al. 1996; SAS Inst. 2002). Fixed effects were year (1 *df*), harvest date within year (4 *df*), species (1 *df*), year × species (1 *df*), and harvest date within year × species (4 *df*). Random effects were replication (2 *df*) and interactions with fixed effects. For all ANOVA, harvest date within year was analyzed as a repeated measure with a first-order autoregressive covariance structure and restricted maximum likelihood estimation method. Degrees of freedom were calculated by the Satterthwaite approximation method (SAS Inst. 2002). For yield data, tests of significance of mean squares were not affected by *ln*-transformation, so ANOVA was conducted on untransformed data. The IVDMD, TNC, and N data were *ln*-transformed before performing ANOVA. Effects of ANOVA were considered significant at $P \leq 0.05$. Means were considered different at $P \leq 0.05$ using the Tukey honest significant difference test (HSD). The MTT data were compared by standard error of the mean ($n = 3$).

Results

At planting (February 2002), tree height (±standard error) was 54.1 ± 0.78 and 33.4 ± 0.96 cm for black locust and mimosa, respectively. Tree basal diameter was 3.9 ± 0.10 and 4.4 ± 0.09 mm for black locust and mimosa, respectively. Thus, black locust seedlings were initially taller and had greater basal stem diameter than mimosa, consistent with a 1-year age difference. However, the age difference did not confound subsequent yield of stem mass tree^{-1}. Preliminary data indicated that pre-test dry matter yield of dormant-pollarded mimosa stems tree^{-1} was relatively constant in 2003 and 2004 (about 32 g tree^{-1}) and did not differ from that of black locust in 2003 (51 g tree^{-1}). However, yield of black locust stems more than doubled to 129 g tree^{-1} from 2003 to 2004 (data not shown). Thus, black locust grew faster than mimosa during the 2-year establishment period.

Leaf yield components

Harvest within year × species interactions for leaf mass shoot^{-1} and leaf mass tree^{-1} were caused in both cases by inconsistent species variation for harvests within year. Leaf mass shoot^{-1} was greater in July and September 2004 (≥29.7 g shoot^{-1}) than the other months within year (14.8–18.7 g shoot^{-1}) when averaged across species. Yield of leaf mass tree^{-1} was low for mimosa in 2005 (Fig. 2). Black locust yielded more than mimosa (153.0 and 126.4 g leaves tree^{-1}, respectively) when averaged across harvests and year. Black locust had greater projected yield (1,900 kg leaves ha^{-1}) than mimosa (1,600 kg leaves ha^{-1}) for a population of 12,300 trees ha^{-1}.

The harvest within year × species interaction showed that mimosa consistently had greater mass leaf^{-1} than black locust in July and August (both years) and in September 2005 (Fig. 3). Mimosa had nearly three times the mass leaf^{-1} as black locust, 2.8

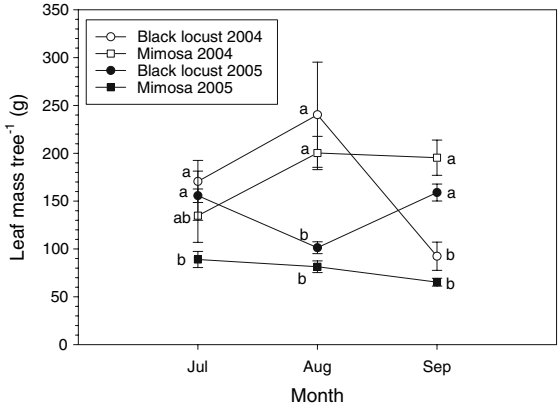

Fig. 2 Leaf mass tree^{-1} of black locust and mimosa harvested in July, August, or September 2004 and 2005 at Booneville, Arkansas. Means within months assigned a common letter do not differ by Tukey's HSD ($P \leq 0.05$). Vertical bars indicate standard error of the mean ($n = 6$)

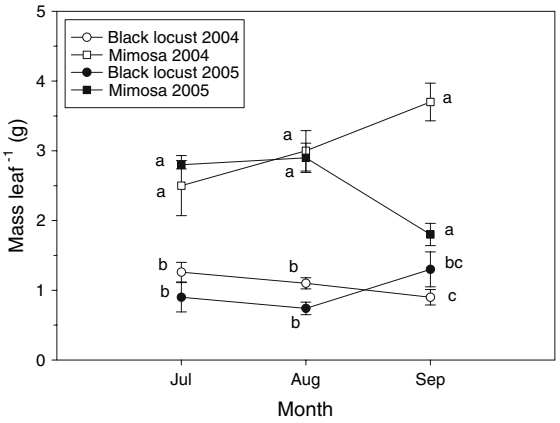

Fig. 3 Mass leaf^{-1} of black locust and mimosa harvested in July, August, or September 2004 and 2005 at Booneville, Arkansas. Means within months assigned a common letter do not differ by Tukey's HSD ($P \leq 0.05$). Vertical bars indicate standard error of the mean ($n = 6$)

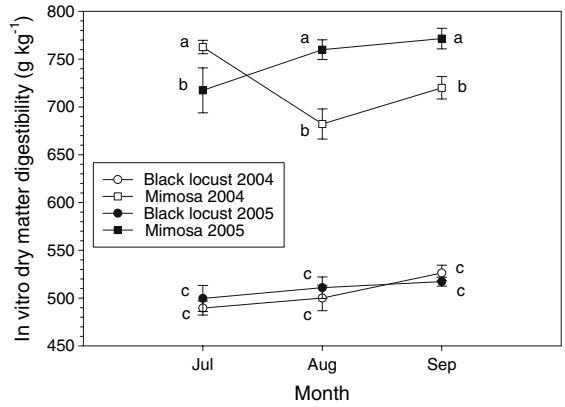

Fig. 4 In vitro dry matter digestibility of black locust and mimosa leaves harvested in July, August, or September 2004 and 2005 at Booneville, Arkansas. Means within months assigned a common letter do not differ by Tukey's HSD ($P \leq 0.05$). Vertical bars indicate standard error of the mean ($n = 6$)

and 1.0 g leaf^{-1}, respectively. Black locust had more leaves (38.8 and 19.2 leaves shoot^{-1}, respectively), more shoots (8.5 and 5.6 shoots tree^{-1}, respectively), more shoot mass (197.5 and 76.5 g tree^{-1}, respectively), and greater basal area (426.1 and 261.4 mm^2 tree^{-1}, respectively) than mimosa. Black locust also produced more biomass tree^{-1} than mimosa (350.5 and 202.7 g tree^{-1}, respectively), but this was mainly due to greater shoot mass than leaf mass tree^{-1}. Projected total (leaf and shoot) biomass yield was greater for black locust (4,300 kg ha^{-1}) than mimosa (2,500 kg ha^{-1}).

Leaf nutritive value

The harvest within year × species interaction indicated that despite small monthly variations, mimosa leaves were highly digestible (range 684–771 g kg^{-1}), consistently more so than black locust (range 491–528 g kg^{-1}) (Fig. 4). There were month within year × species interactions for N and TNC, but no consistent species trends. Black locust had more leaf N (31.2 and 27.1 g kg^{-1}, respectively), and about one-half the leaf TNC than mimosa (27.8 and 59.7 g kg^{-1}, respectively). The month within year × species interaction for TNC was caused by seemingly erratic, possibly spurious, spikes in starch concentration in mimosa leaves which occurred in July 2004 (47.5 g kg^{-1}) and August 2005 (15.1 g kg^{-1}) compared to other month within year × species combinations (range 1.1–4.6 g starch kg^{-1}). The month × species interaction for N digestibility was caused by changes in the magnitude of species differences (data not shown). For any given month in 2004, N digestibility of black locust (49.6–57.3%) was much less than that of mimosa (84.4–92.6%).

Robinin was detected in the standard (Fig. 5a) and in kudzu leaf (0.52 mg mL^{-1}) (Fig. 5d), but concentrations in black locust leaf and bark (Fig. 5b, c, respectively) were less than the detectable limit of 0.01 mg mL^{-1}. Similarly, mimosine was detected in the standard (Fig. 6a), but concentrations in mimosa leaf and bark (Fig. 6b, c, respectively) were less than the detectable limit of 0.03 mg mL^{-1}.

Black locust bark extract was cytotoxic to cultured monkey cells since the 1:8 dilution inhibited cell viability (Fig. 7). The 1:8, 1:4, and 1:2 leaf extracts of black locust were increasingly dark (data not shown) which may have interfered with the MTT assay. Leaf and bark extracts of mimosa were not toxic to cultured monkey cells.

Discussion

The rainfall distribution at Booneville, Arkansas was typical of many areas in the southeastern USA, in which about 20% (251 mm) of total annual rainfall (1,200 mm) occurs during July through

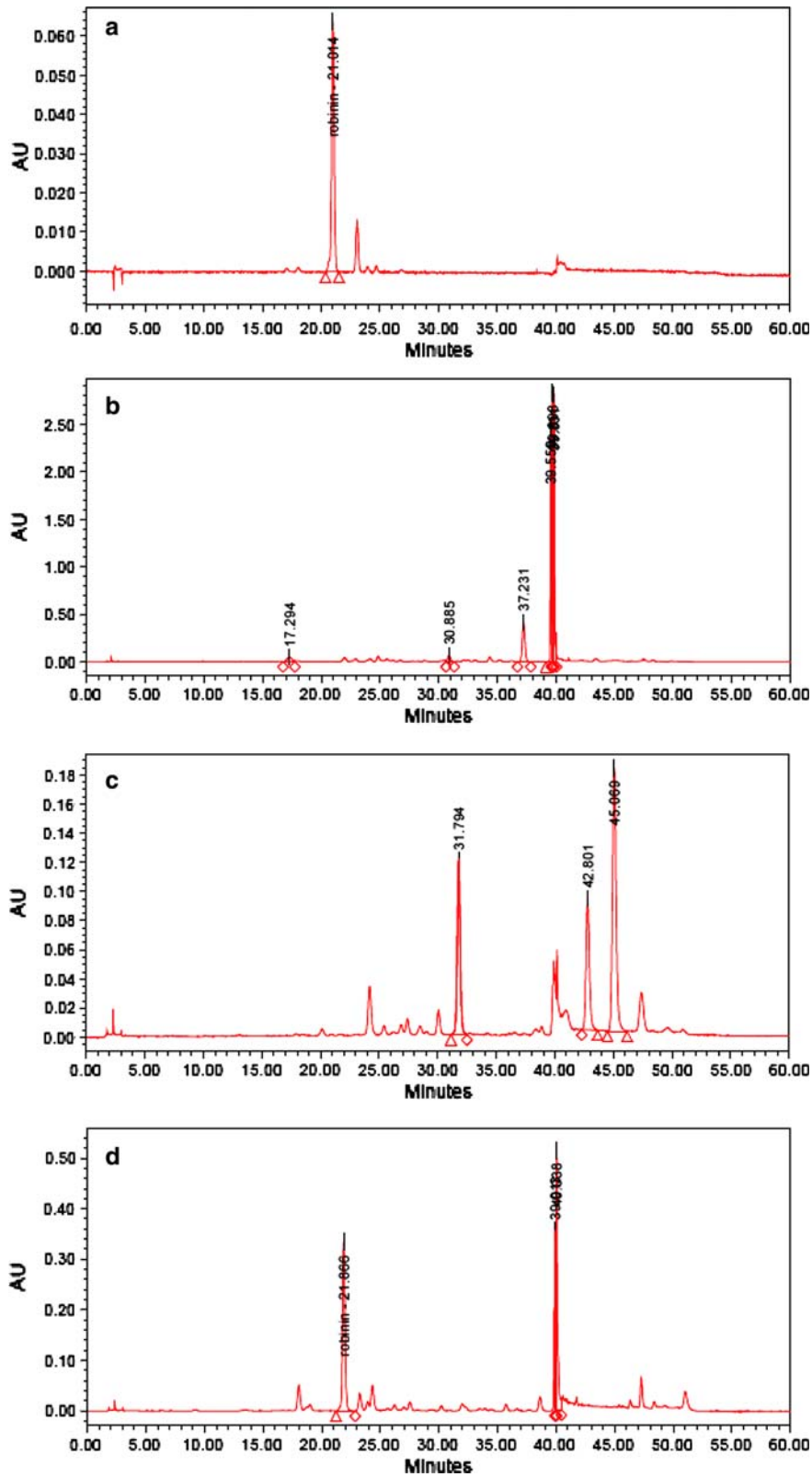

Fig. 5 Chromatograms of **a** robinin standard, **b** black locust leaf extract, **c** black locust bark extract, and **d** kudzu leaf extract. Retention time (min) and absorbance (265.8 and 346.5 nm) units (AU) were *x*- and *y*-axes, respectively. The retention time of robinin was 21.4 min

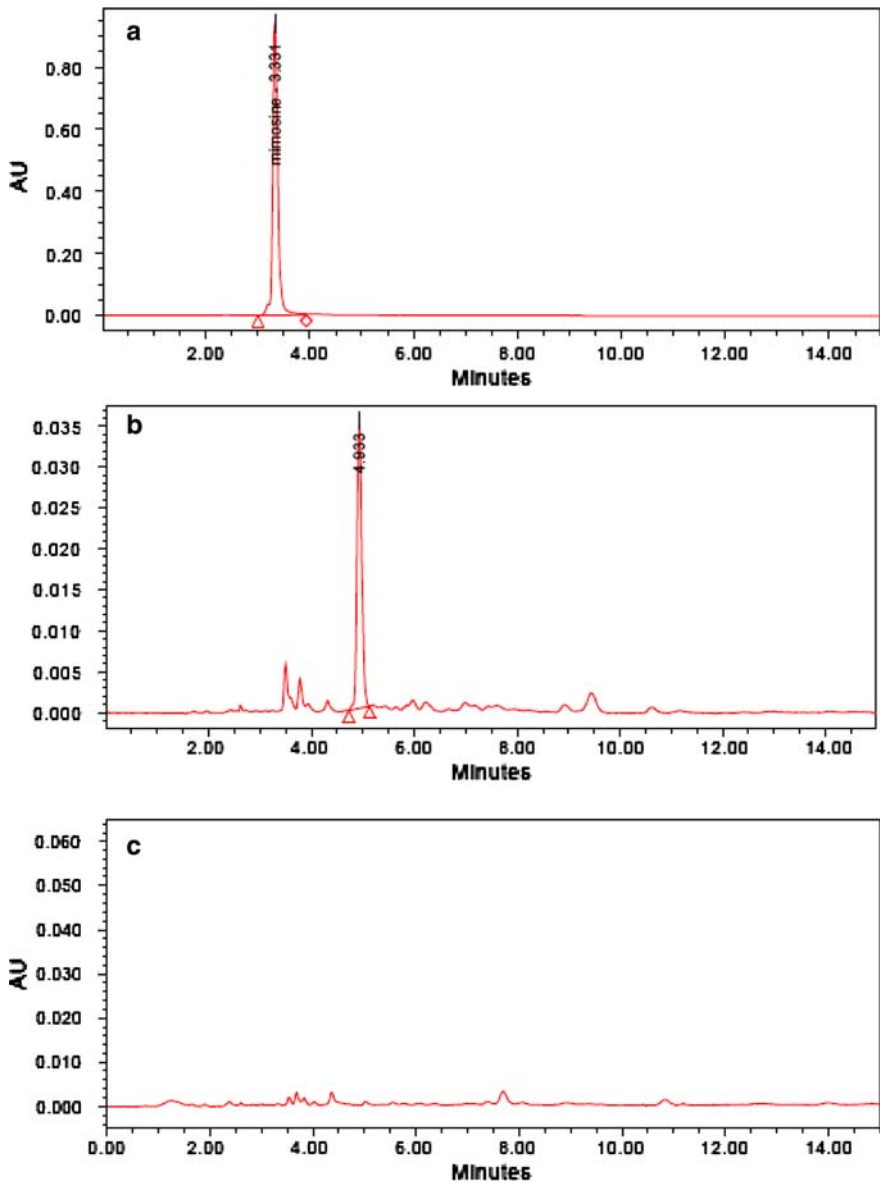

Fig. 6 Chromatograms of **a** mimosine standard, **b** mimosa leaf extract, and **c** mimosa bark extract. Retention time (min) and absorbance (216.4 and 281.2 nm) units (AU) were *x*- and *y*-axes, respectively. The retention time of mimosine was 3.3 min

September when evapotranspiration tends to be high. Relatively low rainfall (especially in August) and high mean air temperature (26°C) occasionally limit the availability of herbaceous forage and livestock feed options during this growth period. Even in semiarid or subhumid regions, woody species are recommended for summer browse because of leaf yield, nutritive value, and ability of certain tree species to withstand repeated defoliation (Papachristou et al. 1999; Papanastasis et al. 1998). Growing conditions (Fig. 1) were sufficiently stressful that black locust typically exhibited premature leaf senescence in late August to early September.

Our first objective was to determine leaf and stem biomass components of pollarded black locust and mimosa. Black locust yielded more leaf mass than mimosa (153.0 and 126.4 g leaves tree^{-1}, respectively), equivalent to theoretical yields of 1,900 and 1,600 kg leaves ha^{-1}, respectively. Black locust also surpassed mimosa for every other yield component (leaves shoot^{-1}, shoots tree^{-1}, shoot mass tree^{-1}, basal area, and biomass tree^{-1}) except mass leaf^{-1}. Leaf yields were less than those previously reported

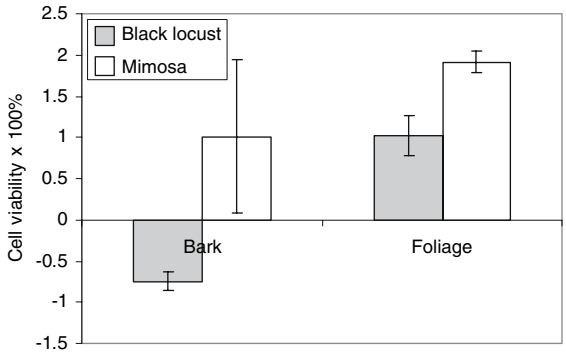

Fig. 7 Viability of cultured African green monkey cells exposed to 1:8 concentration of leaf and bark extracts of black locust and mimosa by the MTT assay. Vertical bars indicate standard error of the mean (n = 3)

(2,390–5,300 kg leaves ha^{-1}) for black locust harvested once per year in North Carolina and Arkansas, USA (Addlestone et al. 1999; Burner et al. 2005). Mimosa yield also was much less than when harvested at 6–8-weeks-intervals (10,460 kg leaves ha^{-1}) in Alabama, USA (Bransby et al. 1995), differences likely influenced by lower rainfall in Arkansas and younger tree age. Natural stands of trees established from rootstock (Burner et al. 2005) are expected to have greater yield potential than transplants, especially if transplants are pollarded, as in this study, sooner than the third year after establishment (Papanastasis et al. 1998). Yield was confounded by cutting frequency because some trees were pollarded during summer and winter each year, while other trees were pollarded only once each year. The effect of this confounding was not measured, but black locust is robust in tolerating repeated, annual pollarding across years (Papanastasis et al. 1998; Snyder et al. 2007). Estimates of leaf and stem mass components (Burner et al. 2006) could be used to calculate cattle stocking and rotation intervals on tree browse under similar densities and management as in this study.

Our second objective was to determine nutritive value of black locust and mimosa leaves for animal browse. The IVDMD, TNC, and N measurements are important predictors of herbage nutritive value, along with ADF and NDF. The IVDMD estimates animal performance by measuring the metabolism of soluble and cell wall constituents (nonstructural carbohydrates, nitrogenous compounds, cellulose, and hemicellulose) (Moore and Hatfield 1994). Mimosa leaves consistently had greater concentrations of IVDMD (range 684–771 g kg^{-1}) than black locust (range 491–528 g kg^{-1}). Concentrations of IVDMD in black locust and mimosa leaves met or exceeded values reported by Papachristou et al. (1999) and Bransby et al. (1995), respectively. Black locust leaf meal (415 g IVDMD kg^{-1}) had about 70% the digestibility of alfalfa meal (605 g kg^{-1}), and the cellulose component of the cell wall was indigestible (Horton and Christensen 1981). Addlestone et al. (1999) reported that black locust leaves had greater concentrations of ADF (320 and 250 g kg^{-1}, respectively) and NDF (500 and 400 g kg^{-1}, respectively) than mimosa, supporting our finding that the potential digestibility of mimosa has better than that of black locust.

Nonstructural carbohydrates are readily fermentable energy sources for rumen micro-organisms, and also are closely related to livestock grazing preference (Mayland et al. 2000). Black locust had about one-half the leaf TNC compared to mimosa (27.8 and 59.7 g kg^{-1}, respectively). Concentrations of leaf TNC have not been previously reported for either species, but were less than (black locust) or equivalent to (mimosa) TNC concentrations of orchardgrass (*Dactylis glomerata* L.) herbage (Burner and Belesky 2004).

Concentrations of leaf N (>27.1 g N kg^{-1}) in black locust and mimosa met or exceeded values reported by Burner et al. (2005) and Cheeke (1992). Feed with 500 g IVDMD kg^{-1} should have a minimum of 71 g CP kg^{-1} (11.4 g N kg^{-1}) for a 250-kg growing beef cow (National Research Council 1996), while a Boer meat goat growing at 0.75 kg day^{-1} needs 113 g CP kg^{-1} (18.1 g N kg^{-1}) at 60% digestible intake protein (Langston University 2000). Thus, total N of black locust and mimosa leaves should be sufficient for growing beef cattle and goats.

The presence of antiquality constituents in black locust leaves is perhaps the greatest constraint to its widespread use as livestock feed. Black locust leaves have 66 g kg^{-1} total phenols (tannic acid equivalents), about the same as *L. leucocephala* (64 g kg^{-1}), but tannin activity [2.2 mg bovine serum albumin (BSA) precipitated mg^{-1} phenol] was greater than that of *L. leucocephala* (0 mg BSA precipitated mg^{-1} phenol) (Makkar and Becker 1998). Tannins are negatively correlated (r^2 = 0.65) with IVDMD (Kamalak et al. 2004). It is not known whether

mimosa leaves contain tannins. Nitrogen digestibility indicates the extent to which tissue N is available to ruminants and, indirectly, the presence of phenolic compounds (e.g., condensed tannins) that block N digestion in the rumen (Cheeke 1992). Black locust leaves had about 17 g digestible N kg^{-1} (at 53% N digestibility), which would meet the nutritional requirements of growing cattle but not growing goats. Nitrogen digestibility was comparable to that reported previously (Burner et al. 2005). Mimosa had about 24 g digestible N kg^{-1} (at 90% N digestibility), which exceeded the needs of growing cattle and goats. Assuming there is a strong predictive relationship between N digestibility and tannin concentrations, black locust leaves could contain about twice the tannins as mimosa.

Our third objective was to determine quantities of the secondary metabolites robinin (black locust) and mimosine (mimosa). Concentrations of robinin in black locust leaves and bark were less than the detectable limit of 0.01 mg mL^{-1}. This nontoxic flavonoid occurs in *Robinia* spp. flowers (Sando 1932; Maksyutina 1972), but presence in leaves was not previously documented. Black locust biomass would not be an economical source of this value-added antioxidant. Similarly, concentrations of the mimosine in leaves and bark of mimosa were less than the detectable limit of 0.03 mg mL^{-1}. Thus, mimosa was unlike *Leucaena* spp. (Soedarjo et al. 1994) in its lack of leaf mimosine. Our data do not agree with the anecdotal evidence that mimosa contains mimosine (Williams et al. 2000; R. D. Williams, 2006, personal communication). Mimosine in *L. leucocephala* decreases in concentration with leaf age, from 45 g kg^{-1} in 1-week-old to 2 g kg^{-1} in 10-week-old leaves (Tangendjaja et al. 1986). Trees in this study were relatively young, and it is not known if concentrations of secondary metabolites could be affected by tree age or leaf sampling outside the July–September period.

Our fourth objective was to assess the in vitro leaf and bark toxicity of black locust and mimosa bark and leaves. This was an assessment of mammalian cellular toxicity, not necessarily that of ruminant toxicity (Hammond 1995). The MTT data supported previous work that mimosa leaf extracts were not cytotoxic (K. Vaughn, 2006, unpublished data). The MTT analysis showed that black locust bark extract was cytotoxic to cultured cells since the 1:8 dilution inhibited cell viability (Fig. 7). The 1:8, 1:4, and 1:2 dilutions of black locust leaves were increasingly dark-colored, which probably caused false positives by interfering with the absorbance readings of the MTT assay. Plant extracts interact with MTT formazan formation by increasing absorbance (Bruggisser et al. 2002), so we cannot state conclusively that the 1:8 dilution of the leaves was not cytotoxic.

Pods and seeds of at least two *Albizia* species contain an unidentified water insoluble, nonalkaloid constituent that causes cattle toxicity in southern Africa (Basson et al. 1970). A water-soluble lectin, robin, initially discovered in black locust inner bark (Jones et al. 1925), is presumably toxic to cattle, horses, and humans (*Homo sapiens* L.) which consume the plant (Hui et al. 2004). The flavonoid acacetin, obtained from a whole-tree extract of black locust, is cytotoxic to a human tumor cell line (Tian and McLaughlin 2000). The inner bark of black locust contains two different lectins, *RPbAI* (major lectin) and *RPbAII* (minor lectin), but concentrations were not reported by Van Damme et al. (1995). Black locust has been targeted for wholesale eradication on the belief that it is toxic to a broad spectrum of animal species (Govt. of Canada 2006). While this may have some element of truth, this action seems rather extreme because goats prefer and even thrive on black locust browse (Addlestone et al. 1999; Luginbuhl and Mueller 1999; Papachristou et al. 1999). Goats could be used as a prescribed grazing approach to site restoration (Tu et al. 2001).

Virtually no data were available on critical toxicity levels for ruminants, or whether cultural practices might influence lectin concentration (Cheeke 1992). Mature leaves tend to have low concentrations of lectins because of translocation to seeds (Kumar 1991), suggesting that toxins other than lectins may be present in leaves, or that leaf age might affect toxin concentration (Tangendjaja et al. 1986). Black locust leaves also may contain phenolic compounds (e.g., condensed tannins) that reduce protein digestibility and herbage intake (Ayers et al. 1996; Cheeke 1992). Low nutritive value (digestibility, N digestibility, and tannins) and low weight gain have argued against its use as feed for chickens (*Gallus gallus domesticus* Brisson) and rabbits (*Oryctolagus cuniculus* L.) (Cheeke 1992). These antiquality factors could be as important as toxins per se in limiting the widespread utilization of black locust browse.

Without evidence to the contrary regarding animal health, we believe that caution should be exercised in exposing cattle and sheep to black locust browse.

Conclusions

Black locust yielded more leaf mass tree^{-1}, leaves shoot^{-1}, shoots tree^{-1}, shoot mass tree^{-1}, basal area, and biomass tree^{-1} than mimosa, while mimosa had greater mass leaf^{-1} than black locust. Mimosa leaves had better feed value (IVDMD, N digestibility, and TNC) than black locust leaves. The spectrum of secondary metabolites did not include robinin (black locust) or mimosine (mimosa). These species could provide seasonal, annually renewable browse for cattle or goats when high ambient temperatures coupled with low rainfall limit the productivity of herbaceous forage. The study did not address the long-term effects of annual pollarding and browsing on leaf yield or tree viability, which could affect sustainability of the grazing system. The sustainability of browse systems might be favored by rest (fallow) periods every few years to minimize depletion of carbohydrate reserves in stem and root tissues. The extract of black locust bark was toxic to monkey cells, unlike that of black locust and mimosa leaves, indirectly verifying reports of toxicity to monogastric animals. Toxicity to ruminants is less clear. Livestock will sample bark tissues during browsing, creating the possibility of adverse health impacts. While it has been established that goats prefer and thrive on black locust browse, the performance of cattle and sheep has not been documented. Further study is needed to detect and quantify toxic constituents of black locust browse, and the effect on cattle and sheep. Producers should consider mimosa instead of black locust browse if livestock health issues are of concern.

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